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Molecular cloning, expression and regulatory activity of $G\alpha_{11}$- and $\beta\gamma$-subunit-stimulated phospholipase C-β from avian erythrocytes

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A turkey erythrocyte phospholipase C (PLC) has been instrumental in delineating the role of G-proteins in receptor-regulated inositol lipid signalling. This isoenzyme is uniquely regulated both by α-subunits of the Gq family and by G-protein $\beta\gamma$-subunits. A 4819 bp cDNA encoding this PLC has been cloned from a turkey erythrocyte cDNA library. The open reading frame of this cDNA encodes a 1211-amino-acid protein (calculated molecular mass 139050 Da) that contains amino acid sequences of 16 peptides sequenced from the turkey erythrocyte PLC. The predicted sequence of the turkey PLC shows considerable similarity with the sequences of previously cloned members of the PLC-β family, with the highest identity (71 %) shared with PLC-β2 and lesser identities observed with PLC-β1 (49 %), PLC-β3 (46 %) and PLC-β4 (37 %). The largest differences in sequence between the turkey PLC-β and other PLC-β isoenzymes occur in the C-terminal domain and in the region between the X- and Y-domains. The turkey isoenzyme and PLC-β2, which differ in their regulation by G-protein α-subunits, are only 44 % similar across the approx. 400 amino acid residues of the C-terminal domain that has been implicated in αq activation of these proteins. Recombinant turkey PLC-β was purified to homogeneity following expression from a recombinant baculovirus in Sf9 insect cells. The immunoreactivity and mobility on SDS/PAGE of the recombinant enzyme were the same as observed with native turkey erythrocyte PLC-β. Moreover, the catalytic activities of the recombinant enzyme were indistinguishable from those of native turkey erythrocyte PLC-β in assays carried out in the presence of cholate and Ca^{2+}, or in assays of activity after reconstitution with $G\alpha_{11}$ or G-protein $\beta\gamma$-subunits. The turkey PLC-β was more sensitive to activation by $G\alpha_{11}$ than was PLC-β2, and was more sensitive to activation by $\beta\gamma$-subunits than either PLC-β2 or PLC-β1.

INTRODUCTION

The inositol-lipid specific PLC isoenzymes have been divided into PLC-β, -γ, and -δ1 families on the basis of both structural and functional similarities [1,2]. Isoenzymes of the PLC-β family are activated by G-protein-coupled receptors through both pertussis toxin-insensitive and -sensitive pathways. The major mechanism whereby PLC-β isoenzymes are regulated apparently involves activation by α-subunits of the Gq family of pertussis toxin-insensitive G-proteins [3–6]. The physiological relevance of the pertussis toxin-sensitive pathway and the identity of the G-proteins involved are not fully established. However, since G-protein $\beta\gamma$-subunits directly activate certain PLC-β isoenzymes [7–11], promotion of inositol lipid hydrolysis through release of $\beta\gamma$-subunits from G_i or G_o probably accounts for the pertussis toxin-sensitive regulation of PLC.

The turkey erythrocyte is a useful model for the study of G-protein-regulated PLC. The high levels of P_{2Y}-purinergic receptor- and guanine nucleotide-promoted PLC activity retained by membranes prepared from these cells has permitted detailed kinetic analyses [12–14]. Moreover, the availability of large quantities of erythrocytes has facilitated purification of protein components of an inositol lipid signalling pathway from a homogeneous cell type. A 150 kDa PLC (PLC-βt) was purified from a cytosolic fraction prepared from these cells [15] and was demonstrated by membrane reconstitution to be a receptor- and G-protein-regulated enzyme [16]. Peptide sequencing indicated that PLC-βt was a unique enzyme with sequence identity to PLC-β isoenzymes [17]. PLC-βt was used to establish a phospholipid vesicle reconstitution assay for a PLC-activating G-protein that subsequently was purified to homogeneity from turkey erythrocytes [5]. Peptide sequencing of this G-protein and molecular cloning of the avian cDNA unambiguously established this signalling protein to be $G\alpha_{11}$ [18]. Other work has led to molecular cloning and expression of the turkey erythrocyte P_{2Y}-purinergic receptor that activates $G\alpha_{11}$ [19].

Here we report the cloning of cDNA encoding the PLC-β cohort of this three-component inositol lipid signalling cascade. Recombinant PLC-βt (rPLC-βt) has been expressed using the baculovirus expression system and purified to homogeneity, and the equivalence of the activities of PLC-βt and rPLC-βt has been established. Potential differences in the regulatory properties of rPLC-βt, rPLC-β1 and rPLC-β2 have been explored.

MATERIALS AND METHODS

Purification of native proteins

Receptor- and G-protein-regulated PLC-βt was purified from turkey erythrocyte cytosol as previously described in detail [15,20]. Avian $G\alpha_{11}$ was purified from cholate extracts of turkey

Abbreviations used: pfu, plaque-formimg units; PLC, phospholipase C; (r)PLC-βt, (recombinant) turkey erythrocyte PLC-β; RT-PCR, reverse transcriptase-dependent PCR; TFA, trifluoroacetic acid.

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The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under accession no. U49431.

erythrocyte membranes [5,21], and G-protein $\beta\gamma$-subunits were resolved from purified bovine brain G_o/G_i as previously described [22].

Peptide sequencing

The amino acid sequence of purified native PLC-βt was obtained from HPLC-purified peptides derived from two separate preparations. Eight peptide sequences of a tryptic digest of purified PLC-βt were previously reported [17]. Two of these sequences were obtained from a single HPLC reverse-phase column fraction that apparently consisted of a mixture of two peptides. These were reported as (vi) EVVISSEPQTASLA and (vii) SY(T/H)QLPFTXL [17]. Subsequent comparison of these two peptide sequences with the deduced amino acid sequence of the turkey erythrocyte cDNA clone 24B-1A reported in this paper suggests that the underlined amino acids were incorrect in the original report. Modification of the previously reported peptide sequences to EV(T/H)QLPEPQTASLA (Table 1, peptide 7) and SYVISSFTXL (Table 1, peptide 6) would be consistent with the deduced amino acid sequence of clone 24B-1A. Eight additional peptide sequences were obtained from a separate preparation of purified PLC-βt as described below (Table 1, peptides 9–16).

Approx. 200 μg of purified PLC-βt was concentrated to 0.2 ml by rotary evaporation. The protein was denatured and disulphide bonds reduced by incubating for 4 h at ambient temperature in the presence of 6 M guanidine hydrochloride, 10 mM dithiothreitol, 2 mM EDTA and 250 mM Tris, pH 8.5. The protein was carboxymethylated by addition of 0.1 vol. of 200 mM iodoacetic acid, the tube was purged with N_2 gas, and the sample was incubated for 30 min in the dark at ambient temperature. The sample was applied to a Vydac C-4 reverse-phase HPLC column equilibrated in 0.1 % trifluoroacetic acid (TFA) and eluted with a linear gradient of 0.1 % aq. TFA to 0.1 % TFA in acetonitrile/propan-2-ol (3:1, v/v) over 60 min at a flow rate of 1.0 ml/min. The peak fraction of PLC-βt was dried by rotary evaporation, dissolved in 70 μl of 70 % formic acid and treated overnight at room temperature with 20 mg of CNBr. The CNBr cleavage products were dried by rotary evaporation, dissolved in 100 μl of 6 M guanidine hydrochloride, 100 mM Tris, pH 8.5, 100 mM dithiothreitol, and applied to a Vydac C-4 reverse-phase HPLC column equilibrated with 0.1 % TFA. The peptides were eluted with a linear gradient from 0 to 80 % solvent B [acetonitrile/propan-2-ol (3:1, v/v), 0.085 % TFA] over 90 min at a flow rate of 1 ml/min. Two well resolved peptides, 15 and 16 (retention times 70.4 and 75.1 min respectively), were concentrated and the amino acid sequences determined. Material eluting as a broad peak of absorbance from the C-4 column (54.0–57.0 min) was pooled, dried, dissolved in 100 μl of 1 % ammonium bicarbonate and digested for 24 h at 37 °C with 0.01 μg of trypsin. Six tryptic peptides resolved on a Vydac C-18 reverse-phase HPLC column were concentrated by rotary evaporation and sequenced on an Applied Biosystems 475A Protein Sequencer.

Oligonucleotide primers

The following five degenerate inosine-containing oligonucleotide primers were prepared on a Cyclone Plus DNA synthesizer (MilliGen/Bioresearch): P14, ATG(C/A)GICCIGA(A/G)ATIGA(T/C)GA(A/G)ATITTTAC (peptide 14; amino acid residues 4–13; sense); P16, GGITG(C/T)TCITG(T/C)GTIGA(G/A)(C/T)TIGA (peptide 16; amino acid residues 8–16; sense); P15r, AA(T/A)GG(G/A)TCAAA(T/C)TG(T/C)TT(G/A)TC (peptide 15; amino acid residues 3–9; antisense); P4r, IGCIACIGC(A/G)TCIGGIAC(G/A)TA(G/A)TC (peptide 4; amino acid residues 1–8; antisense); Px, TG(A/G)TT(T/C)-TCAAAIGAIA(G/A)IAT (common PLC-β X-domain peptide ILSFENH; antisense). Primers for sequencing or PCR amplification of sequences used as probes were synthesized as perfect match primers (21–24 bases in length).

Preparation of turkey erythrocyte mRNA

Washed packed erythrocytes (approx. 24 ml) were prepared as described previously [14] from 100 ml of adult female turkey whole blood and homogenized with a Brinkman polytron homogenizer in 128 ml of 4.0 M guanidine isothiocyanate, 16.7 mM sodium citrate, pH 7.0, 144 mM β-mercaptoethanol and 0.17 % (v/v) anti-foam A (Sigma). Then 8 ml of 10 % (w/v) sodium laurylsarcosinate was added to give a final concentration of 0.5 %. The homogenate was centrifuged at 4000 ***g*** for 10 min and the supernatant was recovered and layered on 15.5 ml of 5.7 M CsCl, 10 mM EDTA, 16.7 mM sodium acetate, pH 5.0. The samples were centrifuged at 113000 ***g*** for 24 h, and the resultant RNA pellets were dissolved in diethyl pyrocarbonate-treated water. The RNA was further purified by sequential extraction with phenol (water saturated)/chloroform (1:1, v/v) and then with chloroform, and was precipitated with ethanol. The final yield of total RNA was 2.2 mg. Poly(A)$^+$ mRNA was purified on two sequential oligo(dT)–cellulose spin-columns (5 Prime-3 Prime) according to the manufacturer's directions, yielding approx. 150 μg of poly(A)$^+$ mRNA. Total RNA from other turkey tissues was prepared by the method of Chomczynski and Sacchi [23] for use in RNase protection assays.

Reverse transcriptase-dependent PCR (RT-PCR)

Reverse transcription of mature turkey erythrocyte poly(A)$^+$ mRNA was carried out with 1 μg of mRNA primed with either 50 pmol of oligo(dT)$_{16}$ or 300 pmol of a degenerate gene-specific primer using the GeneAmp RNA-PCR kit (Perkin-Elmer Cetus) according to the manufacturer's specifications. The temperature cycles were 42 °C for 60 min, 99 °C for 5 min and 5 °C for 5 min. Following reverse transcription, 300 pmol of gene-specific forward and reverse [oligo(dT)-primed reaction only] primers was added and the cDNA amplified by PCR at a final $MgCl_2$ concentration of 2 mM. The reaction cycles for PCR amplification were 4.5 min at 95 °C, then 35 cycles at 95 °C for 45 s, 37 °C for 30 s and 72 °C for 1.5 min. The extension step of the final cycle was increased to 10 min at 72 °C. RT-PCR products were analysed on agarose gels, and bands of the predicted size were excised from low-melt agarose, ligated into the *Sma*I restriction site of pUC-18K and transformed into competent MC1061 cells for further analysis and double-stranded sequencing.

Construction and screening of the turkey erythrocyte cDNA library

Methylated first-strand cDNA was synthesized from 5 μg of adult turkey erythrocyte poly(A)$^+$ RNA using the λ-ZAP cDNA synthesis kit (Stratagene) according to the manufacturer's protocol. A *Xho*I restriction site was introduced into the 3′ end of the cDNA via a *Xho*I/oligo(dT)$_{18}$ adaptor/primer during reverse transcription of the poly(A)$^+$ RNA. The yield of cDNA following second-strand synthesis was approx. 2 μg, and the cDNA ranged in size from 0.6 to 8.0 kb. *Eco*RI adaptors were blunt-end ligated to the double-stranded cDNA, adaptor ends were phosphorylated and the cDNA was digested with *Xho*I prior to size fractionation through a Sepharose CL-4B column. Approx.

100 ng of the > 1 kb cDNA pool was ligated with *Eco*RI/*Xho*I-digested λ-ZAPII and the library packaged with Gigapack II Gold (Stratagene). The primary library contained 3.6×10^7 plaque-forming units (pfu) that were > 99 % recombinant bacteriophage. A portion of the primary library (1.2×10^6 pfu) was amplified to a final titre of 8.9×10^{11} pfu/ml.

The cDNA library was screened with a probe derived from a pUC18K (pUC18 with the kanamycin phosphotransferase gene in place of the β-lactamase gene) plasmid containing the RT-PCR DNA P16/P4r. This plasmid was used as the template for a second PCR reaction using primers located in the 3′ portion of the X-region and the 5′ portion of the Y-region (corresponding to 24B-1A nucleotides 1619–1642 and 2045–2068 respectively; see Figure 1). The resulting 450 bp PCR product was labelled with [α-^{32}P]dCTP to a specific radioactivity of 1.1×10^9 c.p.m./μg by random priming and used to screen the turkey erythrocyte primary cDNA library (1.2×10^6 recombinant plaques) at high stringency. Hybridization was carried out for 16 h at 42 °C with 150 ng of ^{32}P-labelled probe in 50 % formamide, 6 × SSPE, 0.5 % SDS and 100 μg/ml salmon sperm DNA. Following hybridization, the filters were washed three times at 50 °C for 10 min each in 0.1 × SSC containing 0.1 % SDS. Plaques hybridizing with the probe were identified by autoradiography and purified. pBluescript containing the cDNA clone was rescued from the purified λ phage by *in vivo* excision utilizing the ExAssist/SOLR system (Stratagene) according to the manufacturer's recommendations. The size of each clone was analysed by restriction digestion analysis. One of these clones, 24B-1A (4.8 kb), was chosen for further analysis.

cDNA sequencing

Clone 24B-1A was sequenced manually on both strands from double-stranded plasmid using the Sequenase Version 2.0 DNA sequencing kit (United States Biochemical). Four vector-specific and 33 gene-specific primers (18 sense and 15 antisense) chosen to yield overlapping sequences were prepared on a Cyclone Plus DNA synthesizer (MilliGen/Biosearch). The accuracy of double-stranded sequencing was confirmed by subcloning the 5 kb insert from an *Xba*I/*Xho*I digest of 24B-1A.pBS into the *Xba*I/*Sal*I restriction sites of bacteriophage M13 (both mp18 and mp19) and sequencing the resulting single-stranded DNA with both dGTP and dITP nucleotide mixes. Thus each strand of clone 24B-1A was independently sequenced three times. Overlapping sequences within each strand were assembled into a contiguous file using the MacVector AssemblyLign software, and the sense and antisense strands were compared to yield the final consensus sequence (see Figure 1). Sequence comparisons of the turkey clone 24B-1A with seven other members of the PLC-β family were made with MacVector 4.5 sequencing software and the CD-ROM formatted Entrez:Sequences database (release 17.0; June 15, 1995; National Center for Biotechnology Information, NCBI) on a Power Macintosh 8100.

Ribonuclease protection assays

A 587 bp *Bgl*II/*Eco*RI fragment of cDNA clone 24B-1A (nucleotides 2786–3372) was subcloned into *Bam*HI/*Eco*RI-digested pBluescript II (SK$^-$). The new construct was linearized with *Pst*I (there are two internal *Pst*I restriction sites in the insert), and run-off antisense cRNA transcripts (approx. 370 nt) were generated for use as a radiolabelled probe using T7 RNA polymerase in the presence of [α-^{32}P]CTP. Ribonuclease protection assays were performed with 5 μg of turkey erythrocyte total RNA, 0.7 μg of erythrocyte mRNA or 5 μg of total RNA from other turkey tissues using an Ambion RPA II kit according to the manufacturer's protocol. The reaction products were resolved on a 4 % acrylamide (19:1 acrylamide/bisacrylamide)/8 M urea gel. Autoradiographs of the gel were developed following an overnight exposure.

Expression and purification of recombinant proteins

The 5′ untranslated sequence of the turkey erythrocyte PLC cDNA clone 24B-1A was reduced from 361 to 62 bp by PCR amplification of nucleotides 300–916 of 24B-1A. The upstream primer GAGAGAGAGCGGCCGCAGGAGGAG-TAAACTGTGTGA incorporated a *Not*I restriction site on the 5′ end of the cDNA and hybridized to nucleotides 300–319 of clone 24B-1A (Figure 1). The reverse primer TAATGCTG-CTTCCACCCTCTT, equivalent to nucleotides 896–916 of clone 24B-1A, was located downstream of a unique internal *Bam*HI restriction site (nucleotides 481–486). The 633 bp PCR product was purified over a QIA quick-spin PCR purification column (Qiagen), digested with *Not*I and *Bam*HI, and cloned into *Not*I- and *Bam*HI-digested 24B-1A.pBS. This resulted in the replacement of the 361-base 5′-untranslated sequence with 62 bp of 5′-untranslated sequence. The fidelity of the new construct was confirmed by sequencing the plasmid on both strands. Turkey erythrocyte PLC cDNA was subsequently subcloned from pBS into the *Not*I and *Xho*I restriction sites of the mammalian expression vector pcDNA3 (Invitrogen) and from pcDNA3 into the *Not*I and *Xba*I restriction sites of the baculovirus transfer vector pAcMP2 (Pharmingen). Positive clones were confirmed by restriction digests.

Monolayer cultures of *Spodoptera frugiperda* (Sf9) cells were co-transfected with BaculoGold (Pharmingen) and the 24B-1A.pAcMP2 vector. Recombinant baculovirus was purified through two rounds of plaque purification. Plaques expressing rPLC-βt were identified by immunoreactivity with anti-PLC-βt polyclonal antisera (#246) and by quantification of PLC activity.

A 3.2 litre suspension culture of Sf9 cells (3.9×10^9 cells) adapted to serum-free medium was infected at a multiplicity of infection of 3 with the recombinant baculovirus encoding PLC-βt. Infected cells were maintained in serum-free medium (IPL-41; 1 % lipid concentrate, 4 g/l yeastolate ultrafiltrate, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B) at 27 °C for 48 h. Cells were harvested by centrifugation for 5 min at 500 ***g***. The cell pellet was resuspended in 700 ml of ice-cold lysis buffer containing 10 mM Tris, pH 7.4, 5 mM $MgCl_2$, 2 mM EGTA, 0.2 mM benzamidine, 0.2 mM PMSF, 1 μg/ml pepstatin, 1 μg/ml leupeptin and 1 μg/ml aprotinin. The resuspended cells were homogenized using 10 strokes in a Dounce homogenizer and centrifuged at 100000 ***g*** for 45 min. The supernatant was collected as the cytosolic fraction and rPLC-βt was purified by sequential chromatography through columns of Q-Sepharose FF, hydroxyapatite, heparin–Sepharose 4B, Sephacryl S-300 and Mono-Q FPLC as previously described for PLC-βt [15,20]. rPLC-β1 and rPLC-β2 were purified from Sf9 cells as described previously [24].

PLC assays

Total PLC activity was measured as previously described [15,20]. Briefly, 5 μl of sample was assayed for 5 min at 30 °C in a final volume of 100 μl. The final assay mixture contained 10 mM Hepes, pH 7.4, 120 mM KCl, 10 mM NaCl, 2 mM EGTA, 5.8 mM $MgSO_4$, 0.5 % (w/v) sodium cholate, 2.1 mM $CaCl_2$ to give approx. 100 μM free Ca^{2+}, 50 μM PtdIns(4,5)P_2 and approx. 20000 c.p.m. of Ptd[^{3}H]Ins(4,5)P_2. Reactions were terminated by

addition of 375 μl of chloroform/methanol/HCl (40:80:1, by vol.), 125 μl of chloroform and 125 μl of 0.1 M HCl. [^{3}H]Ins(1,4,5)P_3 released into the aqueous upper phase was quantified in a liquid scintillation counter.

Gα_{11} and $\beta\gamma$-subunits were reconstituted with PLC in unilamellar vesicles composed of a 1:4:1 molar ratio of Ptd[^{3}H]Ins(4,5)P_2/phosphatidylethanolamine/phosphatidylserine using the dialysis protocol described by Boyer et al. [9,25]. The time of incubation and amounts of Gα_{11}, $\beta\gamma$-subunit and PLC are indicated in the Figure legends.

SDS/PAGE and immunoblot analysis

SDS/PAGE was performed on gels consisting of 8.5% acrylamide and 0.23% bisacrylamide. Gels were stained with silver nitrate, or proteins were transblotted on to nitrocellulose and analysed as previously described [17] for immunoreactivity with either rabbit polyclonal antisera 246 raised against purified PLC-βt or with PLC-β1- or PLC-β2-specific peptide antisera [24].

RESULTS

Two independent preparations of purified PLC-βt were subjected to proteolytic cleavage by trypsin, CNBr or CNBr followed by trypsin, and the proteolytic fragments were resolved on an HPLC reverse-phase column. The amino acid sequences of the 16 proteolytic peptides isolated by this procedure are shown in Table 1. Peptide 10, isolated from the double digest of PLC-βt with CNBr and trypsin, corresponds to a portion of peptide 8 that was isolated after cleavage with trypsin alone.

Degenerate oligonucleotide primers were designed based on the amino acid sequence of PLC-βt peptides 4, 14, 15 and 16 (Table 1) and on the amino acid sequence ILSFENH (corresponding to residues 404–410 of human PLC-β2), which is highly conserved among members of the PLC-β family. PCR amplification was carried out using reverse-transcribed adult turkey erythrocyte mRNA as a template and the primer pairs P16/P15r, P16/P4r and P14/Px. DNA fragments of 0.95, 1.35 and 0.57 kb were produced from the three sets of primers. The PCR fragments were subcloned into pUC18K, sequenced, and overlapping sequences were identified. Translation of the 1770 bp composite sequence resulted in an amino acid sequence that exhibited high similarity with amino acid residues 221–802 of bovine PLC-β1 and amino acids 217–804 of human PLC-β2. The predicted amino acid sequence of the PCR-amplified turkey DNA contained the sequences (96% identity) of 10 of the peptides resolved from purified PLC-βt.

A 450 bp DNA fragment (equivalent to nucleotides 1619–2068 of clone 24B-1A; see Figure 1, dotted underline) was amplified from plasmid containing the 1.35 kb P16/P4r PCR product and used as a probe for high-stringency screening of an adult turkey erythrocyte λ-ZAP cDNA library. A total of 24 positive clones of various lengths were isolated. Restriction digests and partial sequencing of 15 of the cDNA clones indicated that they corresponded to the same gene. The nucleic acid and deduced amino acid sequences of a full-length clone for PLC-βt (24B-1A) are illustrated in Figure 1. The salient features include: 4819 bases total length, 361 bases of 5′ untranslated sequence, 3633 bases of an open reading frame encoding 1211 amino acids (calculated molecular mass 139050 Da), and 825 bases of 3′ untranslated sequence. Predicted amino acid sequences corresponding to the 16 peptide sequences obtained from PLC-βt are distributed throughout the deduced sequence, and 149 of the 156 total amino acid residues are identical within the deduced amino acid sequence.

Comparisons of the amino acid sequence deduced from turkey clone 24B-1A were made with seven other members of the PLC-β family. The highest identity (71%) was found with human PLC-β2 (Figure 2, top panel). The order of similarity between PLC-βt and other members of the PLC-β family is: bovine PLC-β1, 49%; human PLC-β3, 46%; *Xenopus* PLC-Xβ, 44%; rat PLC-β4, 37%; *Drosophila* PLC-21, 37%; *Drosophila* Norp A PLC, 32%. The highest identity with the other PLC-β isoenzymes occurs in the X-and Y-domains (64–92%), while the lowest exists in the intervening region between the X- and Y-domains and in the C-terminal region (7–44%). The alignment of PLC-β2 with PLC-βt (Figure 2, lower panel) reveals three regions of striking dissimilarity between them. The first (22% identity) occurs in the 49-amino-acid region flanked by the X- and Y-domains (residues 468–516 of PLC-βt). A 186-amino-acid stretch between residues 822 and 1007 of PLC-βt has 36% identity with PLC-β2, whereas a 66-amino-acid portion of the C-terminal region (1146–1211), which has been implicated as an area important for G-protein regulation of PLC, shows only 15% identity. The last 22 amino acids of the C-terminus of PLC-βt are not present in PLC-β2.

Consensus sites for cAMP-dependent protein kinase phosphorylation are underlined in Figure 2 (lower panel). Six of the sites present in PLC-βt are not found in PLC-β2 (Ser-475, -784, -847, -862, -923 and -1066), and PLC-β2 contains three sites not found in PLC-βt (Ser-256, -884 and -960); the two enzymes share three sites (Ser-252, -599 and -610). Two of the PLC-βt sites (Ser-475 and -923) are notable since corresponding sites are not present in PLC-β2 and hydropathy analysis predicts a high probability of surface exposure of these sites in the tertiary structure of the enzyme. Elevation of cAMP levels in turkey erythrocytes markedly reduces G-protein-activated PLC activity in membranes isolated from these cells (M. C. Galas and T. K. Harden, unpublished work).

Table 1 PLC-βt peptide sequences

The amino acid sequences of HPLC-purified peptides isolated from digests of purified native PLC-βt were determined as described in the Materials and methods section. Peptides 1–8 were isolated from a tryptic digest of PLC-βt and the amino acid sequences were reported previously [17]. Peptides 9–16 were obtained using a second preparation of purified PLC-βt from which peptides 15 and 16 were isolated after CNBr digestion and peptides 9–14 were isolated after double digestion with CNBr followed by trypsin. Underlined amino acids correspond to sequences from which degenerate oligonucleotide primers were designed for RT-PCR. Small capital letters indicate amino acid residues which differ from the deduced sequence of clone 24B-1A. The corresponding amino acid residues in clone 24B-1A are shown

Peptide number	Sequence	24B-1A amino acid residues
1	IPGAEVPR	1182–1189
2	YVFPDGPE	1151–1158
3	VIPIIAVHSGYHTVCL	765–780
4	DYVPDAVA	801–808
5	IVALEEGGK	751–759
6	SYVISSFTXL	568–577
7	EV(T/H)QLPEPQTASLA	883–896
8	GQLSPEGMVXFLCR	284–297
9	FIN KK	244–248
10	GQLSPEGM	284–291
11	DLLGK	453–457
12	ILIK	458–461
13	TFLM	210–213
14	NLCMRPEIDEIFTSHHFK	214–231
15	RPDKQFDPFSVDRIDVVVA	661–679
16	YRQTLLAGCSCVELDC	345–360

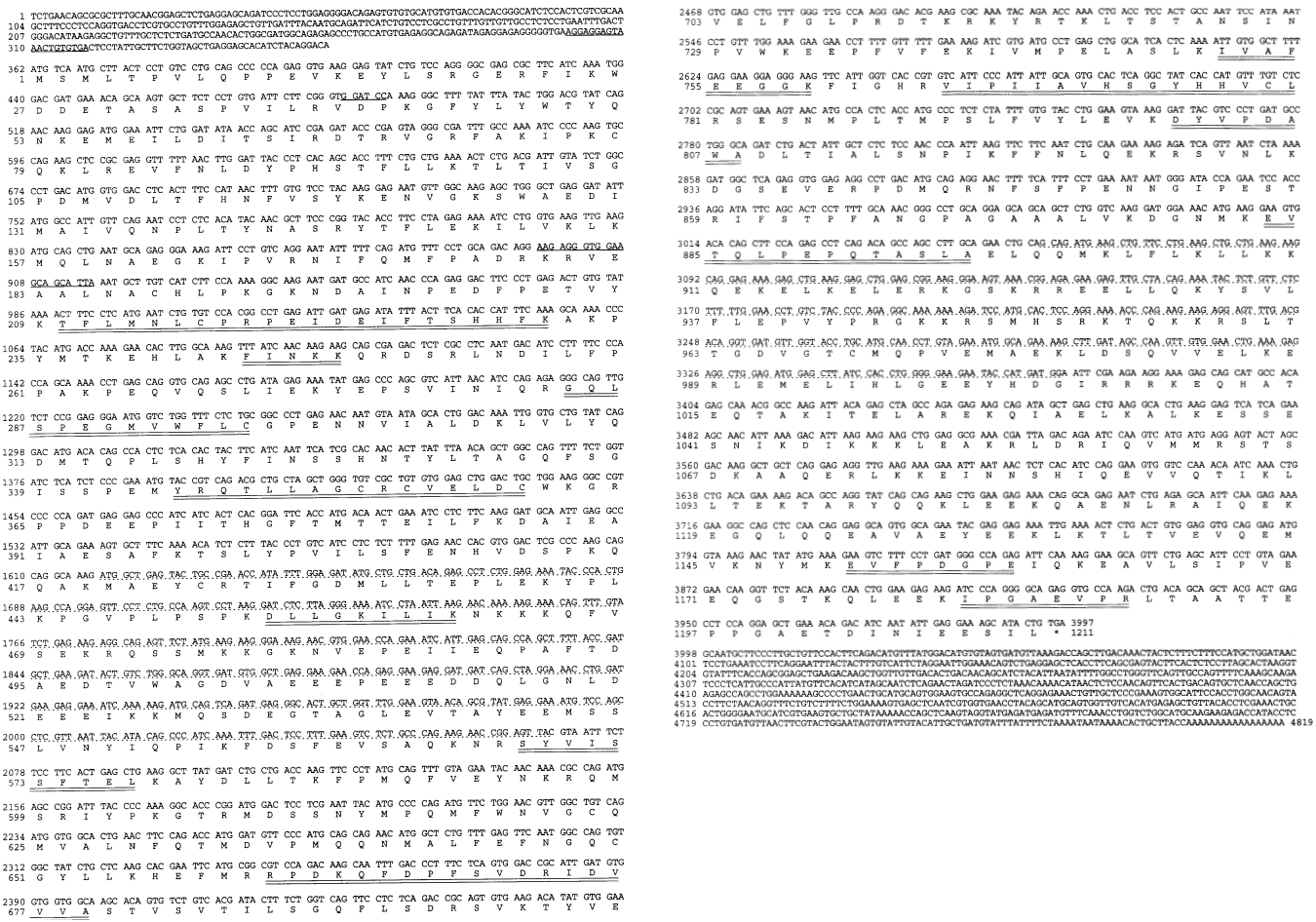


Figure 1 cDNA and deduced amino acid sequences of PLC-βt

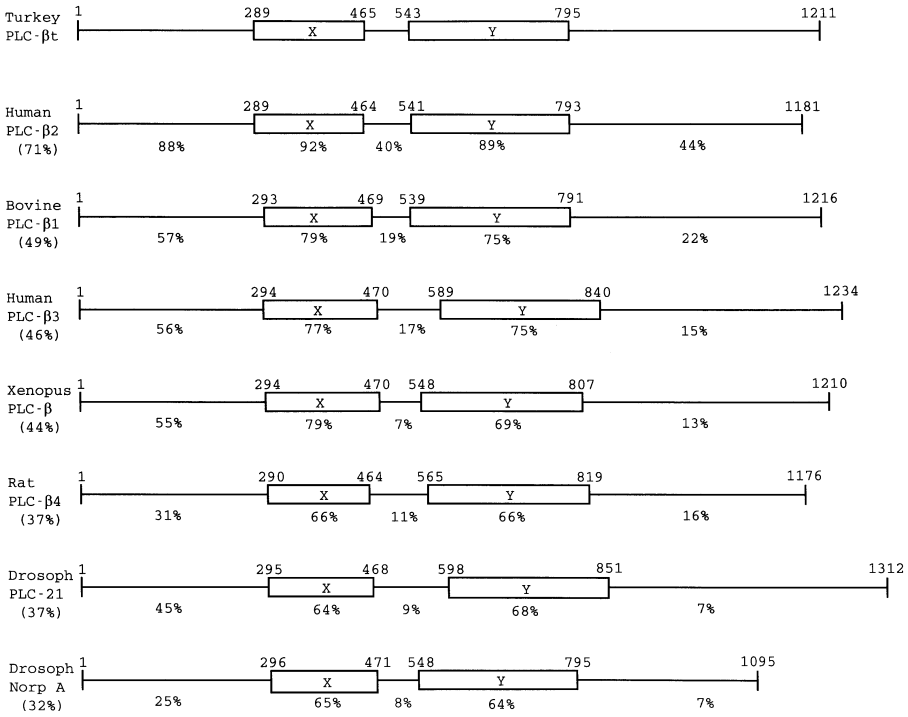
The nucleotide and predicted amino acid sequences of clone 24B-1A are presented. Amino acid sequences corresponding to peptide sequences obtained from purified native PLC-βt are double-underlined. Nucleotide sequence corresponding to the 450 bp probe used for screening the cDNA library (nucleotides 1619–2068) is identified by a dotted underline. cDNA sequence corresponding to the run-off antisense cRNA transcript employed as a probe in RNase protection assays (nucleotides 3058–3372) is identified by a dotted underline. Regions of the nucleotide sequence corresponding to the two PCR primers and the internal *Bam*H1 restriction site used for subcloning 24B-1A into mammalian and baculovirus expression vectors are identified with a single underline (nucleotides 300–318, 896–916 and 481–486 respectively).

The tissue distribution of PLC-βt mRNA was assessed in various turkey tissues using an RNase protection assay (Figure 3). The highest levels of 24B-1A mRNA expression occurred in adult erythrocytes. Much lower levels of mRNA (10% of expression levels in erythrocytes) were observed (in descending order) in lung, kidney, heart, brain and liver. PLC-βt mRNA was not detected in skeletal muscle or intestine.

The baculovirus expression system was used to express rPLC-βt with the goal of confirming that the physical and regulatory properties of the protein encoded by the cloned 24B-1A cDNA correspond to those of the native PLC-βt enzyme. Expression of rPLC-βt, determined by immunoreactivity and enzyme activity (results not shown), was observed within 12–24 h of infection of Sf9 cells and reached maximal levels within approx. 48 h. Approx. 55% of the recombinant protein was recovered in the cytosolic fraction isolated after lysis of infected Sf9 cells.

A cytosolic fraction was prepared from Sf9 cells after 48 h of infection with the recombinant PLC-βt baculovirus, and recombinant enzyme was purified to homogeneity using chromatographic steps previously established for purification of native PLC-βt [15,20]. rPLC-βt exhibited chromatographic properties that were identical to those of the native enzyme. Although rPLC-βt could be purified to homogeneity with fewer chromatographic steps, comparison of the elution properties of the native and recombinant enzymes under these well established conditions provided additional evidence for their similarity. The availability of purified rPLC-β1 and rPLC-β2 also allowed a comparison of the recombinant and native turkey enzymes with other PLC-β isoenzymes. rPLC-βt and PLC-βt co-migrated on SDS/PAGE with a similar mobility to rPLC-β1 and a lower mobility than rPLC-β2 (Figure 4A). The recombinant and native turkey enzymes were immunologically indistinguishable and were distinct from PLC-β1 and PLC-β2 (Figures 4B–4D).

The total enzymic activities of purified PLC-βt and rPLC-βt were compared (Figure 5). Equal molar amounts of each enzyme produced similar rates of hydrolysis of either PtdIns(4,5)P_2 or PtdIns4*P*. Enzyme activities were linear with respect to enzyme concentration (Figure 5, upper panel) and incubation time (results not shown). Each enzyme exhibited a rate of hydrolysis of approx. 15–20 μmol/min per mg when assayed with 200 μM

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PLC-βt  MSMLTPVLQPPEVKEYLSRGERFIKWDDETASASPVILRVDPKGFYLYWTYQNKEMEILDITSIRDTRVGRFAKIPKCQKLREVFN   86
PLC-β2  ..L.N...L..K..A...Q..........TV..........Y.......S....F..........F.K...M..S....D...   86

PLC-βt  LDYPHSTFLLKTLTIVSGPDMVDLTFHNFVSYKENVGKSWAEDIMAIVQNPLTYNASRYTFLEKILVKLKMQLNAEGKIPVRNIFQ   172
PLC-β2  M.F.DNS.......V.....................A....VL.L.KH...A....S...D..........S......K.F..   172

PLC-βt  MFPADRKRVEAALNACHLPKGKNDAINPEDFPETVYKTFLMNLCPRPEIDEIFTSHHFKAKPYMTKEHLAKFINKKQRDSRLNDIL   258
PLC-β2  ............S...................P...S...S.............Y.A...........T....Q........S..   258

PLC-βt  FPPAKPEQVQSLIEKYEPSVINIQRGQLSPEGMVWFLCGPENNVIALDKLVLYQDMTQPLSHYFINSSHNTYLTAGQFSGISSPEM   344
PLC-β2  ....R.D...G..D.....G..A...................S.L.Q...L.HH......N..................L..A..   344

PLC-βt  YRQTLLAGCRCVELDCWKGRPPDEEPIITHGFTMTTEILFKDAIEAIAESAFKTSLYPVILSFENHVDSPKQQAKMAEYCRTIFGD   430
PLC-β2  ...V..S............K..............D.F..E.............P..I...........R..............   430

PLC-βt  MLLTEPLEKYPLKPGVPLPSPKDLLGKILIKNKKKQFVSEKRQSSMKKGKNVEPEIIEQPAFTDAEDTVWAGDVAEEEPEEEDDQL   516
PLC-β2  .........F...........E..R.........N..-.GPTS..KDT.GEA.-GSSPPS.PAVWAGEEGTELEE..VE...EEES   514

PLC-βt  GNLDEEEIKKMQSDEGTAGLEVTAYEEMSSLVNYIQPIKFDSFEVSAQKNRSYVISSFTELKAYDLLTKFPMQFVEYNKRQMSRIY   602
PLC-β2  .....................................T..V...F.....................S.ASV...D.........   600

PLC-βt  PKGTRMDSSNYMPQMFWNVGCQMVALNFQTMDVPMQQNMALFEFNGQCGYLLKHEFMRRPDKQFDPFSVDRIDVVVASTVSVTILS   688
PLC-β2  ..................A.............L.......V......S................N.............T.L.I.VI.   686

PLC-βt  GQFLSDRSVKTYVEVELPGLPRDTKRKYRTKLTSTANSINPVWKEEPFVFEKIVMPELASLKIVAFEEGGKFIGHRVIPIIAVHSG   774
PLC-β2  .....E...R...........G.P..R.....SPST.................L.......RVAVM...N..L...I...N.LN..   772

PLC-βt  YHHVCLRSESNMPLTMPSLFVYLEVKDYVPDAWADLTIALSNPIKFFNLQEKRSVNLKDGSEVERPDMQRNFSFPENNGIPESTRI   860
PLC-β2  ...L..H..........A..IF..M...I.G......V..A......SAHDTK..K..------.AMGGLPEKP..LASPVASQVNG   853

PLC-βt  FSTPFANGPAGAAALVKDGNMKEVTQLPEPQTASLAELQQMKLFLKLLKKQEKELKELERKGSKRREELLQKYSV-L-FLEP--VY   942
PLC-β2  ALA.TS..SPA.R.GAREEA...---AA..R....E..REL.GVV..QRRH....R....R.AR.W.....RGAAQ.AE.G.PG.G   936

PLC-βt  PRGKKRSMHSRKTQKKRSLTTGDVGTCMQPVEMAEKLDSQVVELKERLEMELIHLGEEYHDGIRRRKEQHATEQTAKITELAREKQ  1028
PLC-β2  GV.ACKLGPGKGSR.....-PREESAGAA.G.GP.GV.GR.R...D...L..LRQ...QYECVLK.....VA..IS.MM.......  1021

PLC-βt  IAELKALKESSESNIKDIKKKLEAKRLDRIQVMMRSTSDKAAQERLKKEINNSHIQEVVQTIKLLTEKTARYQQKLEEKQAENLRA  1114
PLC-β2  A........T..NDT.EM.....T...E...G.TKV.T..M......R............V..QM..NLE.H.E.......AC.EQ  1107

PLC-βt  IQEKEGQLQQEAVAEYEEKLKTLTVEVQEMVKNYMKEVFPDGPEIQKEAVLSIPVEEQGSTKQLEEKIPGAEVPRLTAATTEPPGA  1200
PLC-β2  .R.M.K.F.K..L....ARM.G.EA..K.S.RACLRTC..SEAKDKP.RACEC..-P.LCEQDP.IA.-AD.QES..*          1181

PLC-βt  ETDINIEESIL*                                                                            1211
PLC-β2
```

Figure 2 Alignment of PLC-βt with other PLC-β isoenzymes

PLC-β isoenzymes were aligned as described in the Materials and methods section. Upper panel: linear schematic representation comparing PLC-βt with seven other PLC-β isoenzymes. Open boxes represent the highly conserved X- and Y-domains. Amino acid number is indicated above each diagram, and the percentage sequence identity with the corresponding regions of PLC-βt is indicated below each diagram. The overall percentage identity with PLC-βt is indicated in parentheses on the left. Drosoph = *Drosophila*. Lower panel: comparison of the PLC-βt amino acid sequence deduced from the turkey erythrocyte cDNA clone 24B-1A with human PLC-β2. Dots (.) indicate amino acid identity with PLC-βt. The hyphens represent gaps introduced to improve alignment. Consensus phosphorylation sites for cAMP-dependent protein kinase are double-underlined. The asterisk (*) indicates the stop codon.

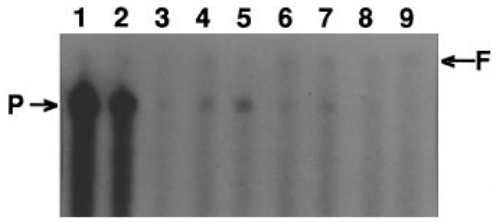

Figure 3 Turkey tissue distribution of 24B-1A mRNA

The tissue distribution of 24B-1A mRNA was determined by RNase protection analysis using a ^{32}P-labelled 24B-1A antisense cRNA probe (full length, 370 bases; protected length, 315 bases) as described in the Materials and methods section. A 5 μg sample of total RNA was used from each tissue, except where noted. The reaction products were resolved on a 4% acrylamide/8 M urea gel, and an autoradiograph of the gel is shown. The lanes contain: 1, adult erythrocyte total RNA; 2, adult erythrocyte mRNA (0.7 μg); 3, brain; 4, heart; 5, lung; 6, liver; 7, kidney; 8, intestine; 9, skeletal muscle. The mobilities of the full-length probe (F) and the protected probe (P) are indicated.

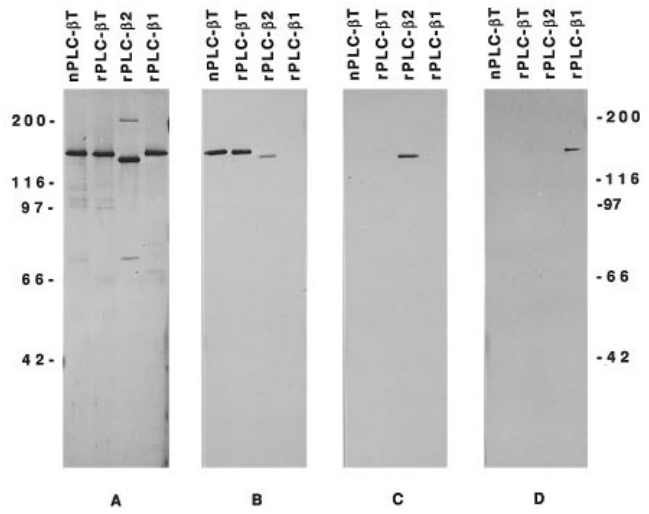

Figure 4 Comparison of purified native PLC-βt, rPLC-βt, rPLC-β2 and rPLC-β1 by immunoblot analysis and silver staining

A sample of 60 ng of each purified PLC-β isoenzyme was subjected to SDS/PAGE and stained with silver nitrate (**A**) or transferred to nitrocellulose (**B**–**D**) and immunoblotted with polyclonal antisera 246 raised against purified native PLC-βt (**B**), PLC-β2-specific peptide antisera (**C**) or PLC-β1-specific peptide antisera (**D**). The mobilities of molecular mass markers (kDa) are indicated on each side.

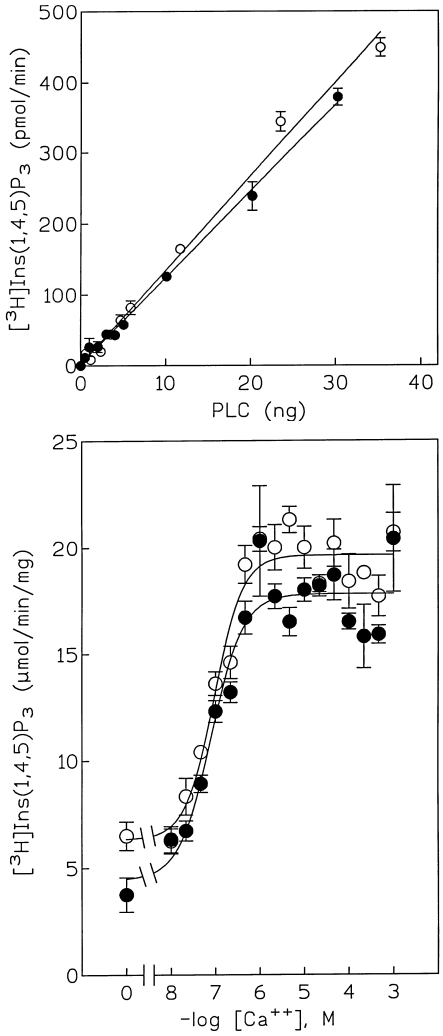

Figure 5 Comparison of total catalytic activities of native PLC-βt and rPLC-βt

Upper panel: the indicated concentrations of purified native (●) and recombinant (○) PLC-βt enzymes were assayed for 5 min at 30 °C using mixed detergent and phospholipid micelles as described in the Materials and methods section. Final reagent concentrations included: 100 μM free Ca^{2+}, 50 μM [^{3}H]PtdIns(4,5)P_2 and 0.5% sodium cholate. Lower panel: samples of 14 ng of purified native (●) or recombinant (○) PLC-βt were assayed for 4 min at 30 °C in the presence of mixed detergent and phospholipid micelles containing (final concentrations) 200 μM Ptd[^{3}H]Ins(4,5)P_2, 0.5% sodium cholate and the indicated concentrations of free Ca^{2+}.

substrate. Both enzymes also exhibited a similar dependence on free Ca^{2+}. Half-maximal activity was observed at 0.2 μM Ca^{2+}, and maximal activity was observed at approx. 1–3 μM Ca^{2+} (Figure 5, lower panel).

Activation of PLC-βt and rPLC-βt by $G\alpha_{11}$ occurred over the same range of $G\alpha_{11}$ concentrations, and similar levels of maximal activation were observed (Figure 6, left panel). G-protein $\beta\gamma$-subunit also activated rPLC-βt to the same extent and over the same range of $\beta\gamma$-subunit concentrations as observed for native PLC-βt (Figure 6, right panel). As previously demonstrated for the native enzyme, approx. 30-fold higher concentrations of $\beta\gamma$-subunit than of $G\alpha_{11}$ were required for activation.

Activation of native and rPLC-βt by either $G\alpha_{11}$ or $\beta\gamma$-subunit was also compared with activation of rPLC-β1 and rPLC-β2 (Figure 7). The regulated activities of the native and recombinant turkey enzymes were indistinguishable at three different concentrations of enzyme. Under these conditions the turkey enzymes exhibited $G\alpha_{11}$-stimulated activities that were approx. 5-fold higher than that of PLC-β2 and 2-fold lower than that of PLC-β1 (Figure 7, upper panel). Similar results were obtained in experiments in which the concentration of PLC-βt, PLC-β1 or PLC-β2 was held constant and the concentration of $G\alpha_{11}$ was varied over a 100-fold range (results not shown). Activities of PLC-βt observed in the presence of G-protein $\beta\gamma$-subunit were approx. 2-fold greater than for rPLC-β2 (Figure 7, lower panel). Similar results were obtained in experiments in which the concentration of PLC-βt or PLC-β2 was held constant and the concentration of $\beta\gamma$-subunit was varied over a 100-fold range

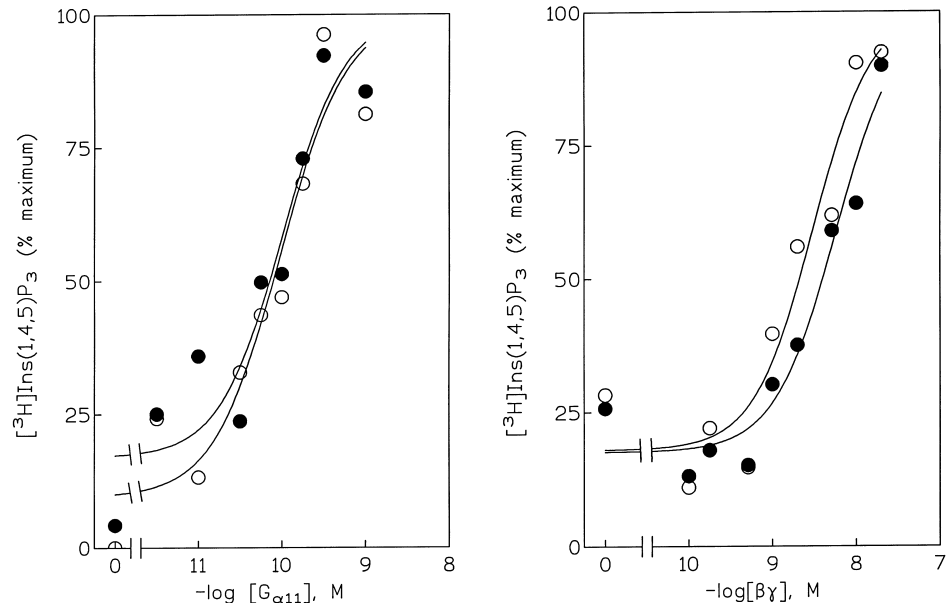

Figure 6 $G\alpha_{11}$ and G-protein $\beta\gamma$-subunit regulation of purified native PLC-βt and rPLC-βt

Left panel: concentration-dependence of purified native turkey erythrocyte $G\alpha_{11}$ for activation of native and recombinant PLC-βt. The indicated final concentrations of $G\alpha_{11}$ were reconstituted with 10 ng of PLC-βt (●) or rPLC-βt (○) using the dialysis protocol described in the Materials and methods section. Assays were for 7 min at 30 °C in the presence or absence of 20 μM $AlCl_3$ and 10 mM NaF (AlF_4^-). The data are the means of two experiments, expressed as a percentage of the maximum AlF_4^--stimulated PLC-βt activity (31 pmol/min). Right panel: concentration-dependence of purified native bovine brain G-protein $\beta\gamma$-subunit for activation of native and recombinant PLC-βt. The indicated final concentrations of $\beta\gamma$-subunit were reconstituted with 10 ng of PLC-βt (●) or rPLC-βt (○) using the dialysis protocol described in the Materials and methods section. Assays were for 7 min at 30 °C. The data are the means of two experiments, expressed as a percentage of the maximum $\beta\gamma$-stimulated PLC-βt activity.

(results not shown). As we and others have previously demonstrated [8,9,11,25,26], $\beta\gamma$-subunit had little effect on the activity of rPLC-β1 under any of these conditions.

DISCUSSION

We have isolated a cDNA from a turkey erythrocyte cDNA library that encodes a 139 kDa protein exhibiting sequence similarity with PLC isoenzymes of the β-isotype. Comparison of the predicted amino acid sequence of this cDNA with amino acid sequence generated from the purified native protein confirmed that the cloned cDNA encodes the PLC-β isoenzyme studied extensively in the turkey erythrocyte model. PLC-βt was expressed in Sf9 cells after construction of a baculovirus expression vector, the recombinant protein was purified to homogeneity, and the physical, catalytic and regulatory properties of this protein were directly compared with those of native PLC-βt purified from turkey erythrocytes. The elution properties of the recombinant and native isoenzymes were identical during chromatography with five different matrices, and recombinant and native PLC-βt shared immunoreactivity against rabbit polyclonal antisera raised against purified PLC-βt. Moreover, the catalytic activity of the recombinant and native enzymes with PtdIns(4,5)P_2 as substrate in the presence of cholate, their activation by Ca^{2+} and their regulated activities upon reconstitution with $G\alpha_{11}$ and $\beta\gamma$-subunits were indistinguishable. Thus a cDNA encoding the turkey erythrocyte PLC-β has been cloned, and as we have previously found to be the case with mammalian PLC-β1 and PLC-β2 [24], the baculovirus/Sf9 cell expression system can be utilized to produce large amounts of homogeneous rPLC-βt that is apparently identical to the native protein.

The turkey erythrocyte enzyme is less than 50 % similar at the amino acid level to mammalian PLC-β1, PLC-β3 and PLC-β4, to *Drosophila* Norp A and PLC-21, and to *Xenopus* PLC-βX, indicating that PLC-βt does not represent a species homologue of these previously cloned PLC-β isoenzymes. In contrast, the 69–71 % identity of PLC-βt with mammalian PLC-β2 places the turkey erythrocyte enzyme at least within the lower range of identities encountered with species homologues. We have isolated cDNAs encoding the other two protein cohorts of the turkey erythrocyte inositol lipid signalling cascade, and these cDNAs predict proteins that are approx. 98 % ($G\alpha_{11}$ [18]) and 88 % (P_{2Y}-purinergic receptor [19]) identical to their mammalian counterparts. We also have isolated a partial cDNA for turkey PLC-β1 (G. L. Waldo, unpublished work) that predicts an amino acid sequence that is 96 % and 94 % identical to those of rat PLC-β1 and bovine PLC-β1 respectively. PLC-βt and mammalian PLC-β2 are only 75 % identical over the analogous amino acid sequence, and the identity of PLC-βt with other PLC-β isoenzymes is less than 60 % in this region. Relatively little is known about the potential existence of splice variants of PLC-β isoenzymes. However, nothing in our work discounts the possibility that turkey erythrocytes express a splice variant of PLC-β2 that has not been previously recognized in other species. Isolation of cDNA that clearly encoded turkey PLC-β2 or that clearly encoded a mammalian homologue of the turkey erythrocyte enzyme will be necessary to resolve the question of equivalence of the cloned turkey erythrocyte enzyme with mammalian PLC-β2. Perhaps the conundrum of their equivalence is a moot question, in that PLC-βt and mammalian PLC-β2 exhibit different properties. PLC-β2 does not co-migrate with the turkey enzyme during SDS/PAGE, and PLC-β2 and PLC-βt exhibit

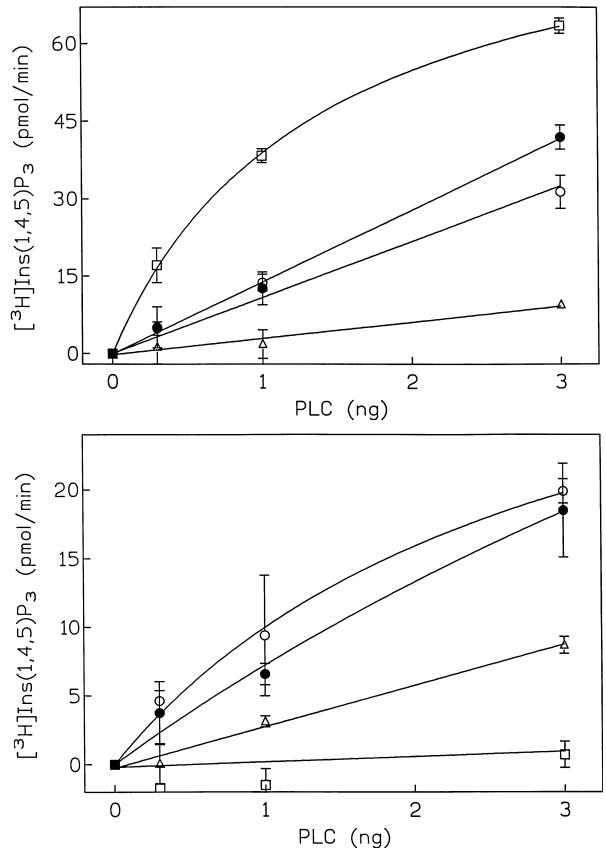

Figure 7 Comparison of Gα_{11} and G-protein $\beta\gamma$-subunit regulation of native PLC-βt, rPLC-βt, rPLC-β1 and rPLC-β2

The indicated concentrations of purified PLC-βt (●), rPLC-βt (○), rPLC-β1 (□) or rPLC-β2 (△) were reconstituted in unilamellar vesicles with 0.3 nM Gα_{11} (upper panel) or 10 nM $\beta\gamma$-subunit (lower panel) using the dialysis protocol described in the Materials and methods section. Assays of Gα_{11}-promoted activation were in the presence or absence of AlF_4^-, and AlF_4^--stimulated activity is shown (upper panel). All assays were for 5 min at 30 °C.

different immunoreactivities. Moreover, PLC-βt is activated by lower concentrations of Gα_{11} compared with those necessary for activation of PLC-β2, and PLC-βt is more sensitive than either mammalian PLC-β1 or PLC-β2 to activation by $\beta\gamma$-subunit.

Overall percentage identities among the PLC-β isoenzymes may be less important than identities in specific regions, since the highly conserved X- and Y-domains comprise such a large proportion of PLC isoenzymes. Since the domains responsible for G-protein α- and $\beta\gamma$-subunit activation have not been unambiguously defined, we can only speculate on the significance of marked divergences in sequence from other PLC-β isoenzymes found in regions outside the X- and Y-domains of PLC-βt. Several lines of evidence suggest that activation of PLC-β isoenzymes by α-subunits of the Gq family of G-proteins occurs primarily through interactions in the C-terminal domain of these enzymes [27–29]. Thus it may prove useful to establish whether the C-terminal extension found in the turkey enzyme plays a role in differences in the α-subunit regulation of PLC-βt compared with PLC-β2, and the functional significance, if any, of divergence in the sequences of PLC-βt and PLC-β2 elsewhere in the C-terminal domain should be established. Little is known about the domain(s) of PLC-β isoenzymes involved in $\beta\gamma$-subunit regulation, although regions outside the C-terminal domain are apparently responsible [29–31]. PLC-βt is one of the most sensitive to $\beta\gamma$-subunit activation of any of the known PLC-β isoenzymes, and availability of cDNA encoding the turkey isoenzyme should now allow us to delineate the domain involved in $\beta\gamma$-subunit regulation in an isoenzyme that is markedly activated by both α- and $\beta\gamma$-subunits. The relative importance of the poorly conserved sequence (residues 468–576) in the domain between the X- and Y-domains is not known, but this sequence in PLC-βt, which differs markedly from sequences in other PLC-β isoenzymes, could eventually provide an insight into the functionality of this domain.

James et al. [32,33] and Boguslavsky et al. [34] have reported that native PLC-βt binds with high affinity to polyphosphoinositide-containing phospholipid vesicles. In contrast, rPLC-β2 and rPLC-β1 exhibit markedly lower binding affinities (S. R. James, A. Paterson, T. K. Harden and C. P. Downes, unpublished work). The availability of cDNA encoding PLC-βt should allow us to begin to address the structural basis of differences observed among these proteins in their capacity to interact with phospholipid membranes and PtdIns(4,5)P_2.

A so-called pleckstrin homology domain has been identified in nearly 100 different proteins [35]. Although the function(s) of this domain has not been unambiguously established, a role in membrane targeting has been strongly implicated. Thus it is not surprising that PLC isoenzymes, including those of the PLC-β class, possess pleckstrin homology domains [36]. The predicted sequence of PLC-βt also includes a pleckstrin homology domain in the N-terminus and thus provides an additional protein sequence in which the function of such a domain and the potential importance of its interaction with G-protein $\beta\gamma$-subunits can be established.

We know from studies with intact erythrocytes that the P_{2Y}-purinergic-receptor-regulated inositol lipid signalling cascade is very sensitive to inhibition by elevation of intracellular cAMP (M. C. Galas and T. K. Harden, unpublished work) and presumably by activation of cAMP-dependent protein kinase. In contrast to PLC-β2, we have found that PLC-βt is also an excellent *in vitro* substrate for cAMP-dependent protein kinase (A. Paterson, G. L. Waldo and T. K. Harden, unpublished work). Several consensus sites for phosphorylation by cAMP-dependent protein kinase are uniquely found in PLC-βt, and we are currently establishing the role of these sites in regulation of enzyme activity.

We have previously purified large amounts (up to 30 mg) of homogeneous rPLC-β1 and rPLC-β2 after baculovirus expression in Sf9 cells [24]. Although we have not yet generated large-scale preparations of homogeneous PLC-βt, the baculovirus expression system apparently works equally well for production of homogeneous turkey enzyme. The fidelity with which rPLC-βt retains the physical and regulatory properties of native PLC-βt supports the idea that baculovirus-promoted expression of PLC isoenzymes in Sf9 insect cells provides an ideal approach for preparation of large amounts of these effector proteins in a homogeneous form. This should prove highly advantageous for the unambiguous definition of α- and $\beta\gamma$-subunit regulatory domains in PLC-βt, for delineation of amino acid residues important in phospholipid surface binding, and for delineation of important phosphorylation sites in this effector protein.

In summary, a cDNA encoding PLC-βt has been cloned and the recombinant protein has been expressed, purified and characterized. We have now isolated the cDNAs for all three protein cohorts of a receptor-regulated PLC of a homogeneous cell, which places us in a position to reconstruct a receptor–G-protein–effector-protein cascade from a single cell type.

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